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(KOREAN HEMORRHAGIC FEVER)

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19. ABSTRACT (Continue on reverse if necessary and identify by block number)

World-wide, about 200,000 people are hospitalized with Hemorrhagic fever with renal syndrome (HFRS) (3-10% fatality) each year. The etiologic agents of HFRS are Hantaan, Seoul and Puumala viruses of the genus Hantavirus, family Bunyaviridae. A severe form of HFRS, caused by Hantaan virus, occurs in Asia and eastern parts of Europe, a moderate form, caused by Seoul and Seoul-like virus, occurs in Asia, and a mild form, caused by Puumala virus, occurs in Europe. The reservoirs of hantaviruses are rodents and small mammals. Geographical distribution of hantaviruses and surveillance of HFRS are important for prevention of this highly fatal disease. Little is known about the pathogenesis of HFRS. It is also important to investigate antigenic differences of hantaviruses isolated from non-endemic areas of the world because HFRS patients have never been documented in many areas despite the finding of positive man and rodents there.

The methods for diagnosis of HFRS, isolation of hantaviruses from man and rodents are described previously. A new high density silicone particles were used for a rapid serologic

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diagnostic test for Puumala virus infection.

Total nos. of confirmed HFRS patients serologically in 1990 and 1991 were 1,043 and 956, respectively and large outbreaks of scrub typhus, murine typhus, leptospirosis and spotted fever occured during epidemic season of HFRS in Korea. Recently, no. of HFRS patient among civilian population is increasing not only in rural areas and also in urban cities. Male is dominant group in HFRS and children are also victims of HFRS. Serologic evidences for hantavirus infection in wild bats and wild birds were demonstrated for the first time in Korea. A simple and rapid serologic diagnostic test for Puumala virus infection was developed by high density silicon particle agglutination. Necrosis and detachment of the epithelial cells of renal tubules and Hantaan virion-like structures in the kidneys of patients who died during the acute phase of HFRS were demonstrated by EM for the first time. It was demonstrated that prototype Hantaan, Puumala viruses and a local strain of hantavirus should be used as antigens for accurate serodiagnosis of HFRS in different parts of the world.

SUMMARY

World-wide, about 200,000 people are hospitalized with Hemorrhagic fever with renal syndrome (HFRS) (3-10% fatality) each year. The etiologic agents of HFRS are Hantaan, Seoul and Puumala viruses of the genus Hantavirus, family Bunyaviridae.

The followings are details of the research for the period February 19, 1990 through October 9, 1991, and summary of the research for the period February 10, 1986-October 9, 1991.

Large outbreaks of scrub typhus and leptospirosis occured during epidemic season of HFRS in 1986 and nos. of confirmed patients serologically at our laboratory were 215 and 64, respectively. It was demonstrated that field mice and wild rats are reservoir hosts of HFRS, scrub typhus and leptospirosis in Korea. Global distribution of hantaviruses was demonstrated by sero-epidemiologic surveys. Five strains of Seoul virus were isolated from urban rats caught in Hong Kong and Singpore for the first time in 1986.

Serologic sruvey of field Apodemus mice against Hantaan virus, R. tsutsugamushi and leptospira infections in the U.S. Marine camp in Wuncheon, Kyunggido in 1987 where exercise "Bearhunt" has been held every year showed high infection rates with the agents. Seoul virus was isolated from a Syrian hamster purchased from a local animal farm in Seoul and it was demonstrated that inbred hamsters are broad spectrum animal model for growth of hantaviruses. Hantavirus infection was demonstrated for the first time among laboratory personnels, laboratory rats and wild M. musculus in Argentina in 1987.

Outbreaks of murine typhus and spotted fever were documented during epidemic season of HFRS 1988 for the first time in Korea and nos. of patients were 448 and 327, respectively. So now we know that HFRS, scrub typhus, murine typhus, spotted fever and leptospirosis are hemorrhagic diseases exist in Korea. Four HFRS patients were documented in Sri Lanka for the first time and a strain of Seoul-like virus was isolated from R. norvegicus caught in Colombo, 1988.

A severe form of HFRS, caused by Hantaan and related viruses, occurs in Asia and eastern parts of Europe, a moderate form, caused by Seoul virus, occurs in Asia, and a mild form, caused by Puumala virus, occurs in Europe. Serological studies of 42 hantaviruses isolated from HFRS patients and from animals in the different parts of the world indicated that there are 6 or 7 serotypes. A simple and rapid serologic diagnostic test for Hantaan virus infection was developed by high density particle agglutination and antibodies against Hantaan and Seoul viruses were measured within 1 hour. Vertical transmission of Hantaan virus in a pregnant woman has been decumented in 1989.

Total number of hospitalized HFRS patients serologically confirmed in Korea in 1990 and 1991 was 1,043 and 956, respectively. No. of civilian HFRS patient in rural areas and in urban cities of S. Korea is increasing every year but no. of HFRS patient among soldiers is decreasing since 1988. Serologic evidences for hantavirus infection in wild bats and wild birds were demonstrated for the first time in Korea, 1990. A simple rapid serologic diagnostic test for Puumala virus infection was developed by high density silicon particle agglutination in 1991. For understanding of pathogenesis of HFRS, thin section EM of the kidneys of patients died during the acute phase of HFRS was applied and occurrence of numerous dense precipitates, typical inclusion bodies, a surface antigen layer, as well as Hantaan virion-like structures were detected in the kidneys. Comparative study of serologic diagnosis of sera from suspect HFRS patients against hantaviruses showed that Hantaan virus, Puumala virus and a local strain of hantaviruses should be used as antigens for correct diagnosis of HFRS in different parts of the world.

In the 1990s, it is highly possible to identify HFRS and HFRS-like illnesses caused by hantaviruses in parts of the world where HFRS is not known because of the availability of serodiagnostic tests.

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FOREWORD

In conducting the research described in this report, the investigators (s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animals Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

TABLE OF CONTENTS

INTRODUCTION

During the Korean War more than 3,200 United Nations troops in Korea developed a rare hemorrhagic fever, a situation that attracted worldwide attention (1). Since then it has been known as Korean hemorrhagic fever (KHF) in Korea. This disease was an important military problem because large epidemics occurred among soldiers during several wars. More than 12,600 cases of epidemic hemorrhagic fever (EHF) occurred among one million Japanese soldiers in Manchuria (2) and several hundred cases among Russian soldiers in the Far East (3) during World War II. Several thousand cases of war nephritis, clinically similar to Nephropathia epidemica (NE), were reported among British soldiers stationed in Flanders during World War I (4), and about 16,000 cases of NE occurred among German soldiers in Lapland and prisoners in Yugoslavia during World War II (5). About 14,000 cases of war nephritis clinically similar to NE were described among Northern Armies in the American Civil War (6).

In South Korea, 500 to 1000 persons are hospitalized annually with this disease and about one third of these were soldiers (7). There were about 114,000 cases of HFRS in China in 1986 with 7% mortality, and several hundred cases of HFRS occurred in other countries of Asia and Europe (8,9).

The causative agent of KHF was first discovered in 1976 from Apodemus mice (10) and isolated from KHF patients in 1978 (11). This agent has been propagated in a human cell culture line (12), and it was named Hantaan virus after the Hantaan river which runs along the 38th Parallel between South and North Korea (13). Antigenic features, genetic properties and EM studies indicate that Hantavirus is a new genus of Bunyaviridae (14-17). A close etiological relationship has been established between KHF and hemorrhagic nephroso-nephritis in the USSR, NE in Scandinavia, EHF in Greece and Eastern Europe, Japan and China (11,18-21).

The working group on HFRS at a WHO meeting in Tokyo, 1982 recommended that all of the above diseases with different names should be referred to as "Haemorrhagic Fever with Renal Syndrome (HFRS)" (22). Recent sero-epidemiologic surveys established that Hantaviruses are widely distributed throughout the world (23-29).

Intraspecific transmission of Hantaan virus in Apodemus mice (30) has also been shown. Infection occurred among cagemates up to 360 days after exposure, while large amounts of virus were excreted in urine and saliva. No evidence for the participation of ectoparasites in virus transmission was found. Infection with Hantaan virus is silent in animals (31), but is associated with diverse clinical symptoms in human (28).

A severe form is common in East Asia, while most European cases are mild (28). The disease is most often sporadic, but under special circumstances epidemics occur. Although predominantly associated with rural areas, HFRS is now being recognized as an urban problem in some countries (28,32,33) and a particular hazard to laboratory staff using rodents for biomedical research (28,34,35). From 1975 to 1986, about 160 cases of HFRS of which one was fatal, occurred in 34 animal rooms of research Institutes in Korea, Japan and Europe among personnel of the animal rooms and researchers as a result of exposure to infected rat colonies. Seventy-one% of Korean rats and 40% of the Japanese rats had antibodies to Hantaan virus (28). Commercial rabbits bought from breeding firms in Korea and Japan were seropositive to Hantaan virus and serum antibodies were found in 3.5% of 792 New Zealand rabbits (36). We have registered a Hantaan related virus isolated from an urban rat caught in Seoul, 1980 as Seoul virus in 1985 (13). Several strains of Seoul virus were isolated from urban rats caught in Korea and Japan and many strains of Hantaan and Seoul virus were isolated from blood of HFRS patients in Vero E6 cell cultures (7,28).

Recently, there have been several outbreaks of acute hemorrhagic diseases among soldiers and farmers before and during the epidemic season of HFRS in Korea and it was confirmed that leptospirosis, scrub typhus, murine typhus, spotted fever and other rickettsiosis are the hemorrhagic diseases existing in Korea (7,37).

An inactivated Hantaan virus vaccine with formalin was developed and field trial of this vaccine showed 99% seroconversion by IF antibody tests after administration of two doses. The vaccine was safe but the efficacy of this vaccine against HFRS in the endemic areas of HFRS remains to be determined (38).

There are still many problems to be answered in research work of HFRS and some important issues are: a) global survey of Hantavirus infection and HFRS b) serologic relation of new hantaviruses isolated from the different parts of the world c) development of a simple serologic diagnostic test d) pathogenesis of hemorrhages and nephritis and e) development of an animal model mimic to man.

This report describes 1) seroepidemiologic survey of HFRS and other hemorrhagic diseases in Korea 2) serologic survey for hantavirus infection of wild bats and birds 3) development of a simple and rapid serologic diagnostic test for Puumala virus infection and 4) pathogenesis of HFRS in man.

MATERIALS AND METHODS

Collection of field and urban rodents

Field and house rodents were captured by means of baited live traps and normal Apodemus mice were captured on Jeju island as described (11,14). Seronegative Apodemus mice and Wistar rats were used as sensitive detectors for Hantavirus isolation.

Processing rodents

Living rodents were identified and bled by cardiac puncture under chloroform anesthesia. Serum was separated for antibody titration. Necropsy tissue include lungs, spleen, liver, and kidneys. A portion of each organ was examined immediately by IFA for Hantavirus antigen and the remaining portion were frozen at -70°C until processing for virus isolation.

Collection of wild bats and birds

Bats and birds were captured by means of nets in the endemic areas of HFRS.

Processing bats and birds

Living bats and birds were identified and bled by cardiac puncture under chloroform anesthesia. Necrospy tissue include lungs, spleen, liver and kidneys. A portion of each organ was examined immediately by IFA for Hantavirus antigen and the remaining portion were frozen at -70°C until processing for virus isolation.

Sera from patients

Sera collected from suspected HFRS patients in Korea and in other countries were used for serodiagnosis.

Viruses

The hantaviruses studied were from HFRS patients and rodents collected in different parts of the world. These were used after propagation in Vero E6 cell cultures. ID_{50} of the viruses in Vero E6 cells was $10^{5.0}$ - $10^{6.0}$ /ml against hantaviruses.

Antisera against hantaviruses

Two 5 week old S.D. rats were immunized with each virus by giving them a single intramuscular inoculation of 0.5 ml supernatant fluid from infected Vero E6 cell cultures. Whole blood was collected by cardiac puncture 28 days after inoculation of the virus and serum was separated from the clot, stored at -70°C, and then tested for antibody against the homologus virus and other hantavirus isolates. Serum samples from convalescent-phase HFRS patients in Korea, Japan, Yugoslavia and Finland, and monoclonal antibodies to Hantaan virus were used for comparative serologic studies of hantaviruses.

Antibodies

Neutralizing (N) antibodies to hantaviruses were measured by plaque reduction neutralization test (PRNT) and monoclonal antibodies to hantaviruses were titrated by indirect immunofluorescent antibody technique (IFAT), as described previously (39).

ELISA test

This test for demonstration of IgG and IgM antibodies against Hantaan and Puumala virus antigen was developed recently, however, it can not differentiate Hantaan virus infection from Seoul virus infection because of cross reaction between them and the method is as described (39).

Antibody test against rickettsiosis

R. tsutsugamushi, R. typhi and R. sibirica strains were obtained from US Army Medical Research Unit in Malaysia. Antigens were prepared in Yolk sac and micro-immunofluorescent antibody technique were used for antibody titration (37).

Carrier particles

High density composite particles (40) (HDP, Tokuyama Soda Co., Tokyo, Japan) were used as carrier. They have a silica core surrounded by a red dye layer and second silica layer covered the dyed layer. Furthermore, the particle surface is covered with functional groups designed to adsorb antigen. The density of the particles is 2.0 and their diameter is 1.8 μm.

Puumala-HDPA antigen

Puumala virus #141247, strain (29), was used in the HDPA experiments. The virus was passaged 14 times in a rodent brains to increase titers and virus yield. The ID_{50} of the virus in a rodent by intracerebral inoculation was $10^{8.2}$ /ml. Supernatant fluid of 5% brain suspension in phosphate buffered saline (PBS), pH 7.2 was inactivated with 0.05% formalin at 4°C for 15 days. Purification of the inactivated virus suspension for use as antigen in HDPA was done according to the modified method used for preparation of Japanese encephalitis mouse brain vaccine (41). Protein content of the purified antigen preparation was 43 μg/ml and antigen concentration of the preparation was 80,000 units/ml by ELISA test.

Preparation of Puumala antigen coated HDP

For preparation of Puumala virus antigen coated HDP, an equal volume of eight ELISA units/ml of Puumala-HDP antigen in PBS was added to the 0.5% HDP suspension in PBS in 1/60 mole, and incubated two hours at 20°C shaking each ten minutes. Then the HDP were washed with PBS twice, suspended in 0.1% of the original volume of PBS containing 0.01% bovine serum albumin, 1% dextran, 1% sodium glutamate and 0.5% glycine as a stabilizer, and then lyophilized (42). Uninfected rodent brains were also treated in same manner as antigen control.

Sera from HFRS patients for Puumala HDPA

Eleven sera from HFRS patients from Korea, Japan and Finland were used for antibody determinations. Antibody negative serum from a healthy person in the U.S.A. was also used as negative control serum. Serum samples from Korean HFRS patients had been shown in other tests to contain antibodies against Hantaan virus. Likewise, serum samples from HFRS patients in Japan were known to contain antibodies to Seoul virus and serum samples from Nephropathia epidemica patients in Finland contained antibodies to Puumala virus.

Procedure of Hantavirus HDPA test

A microtiter technique was used for HDPA test for antibodies against Puumala virus. The virus antigen and normal antigen coated HDP were suspended to a concentration of 0.5% with buffer (42).

Acute phase HFRS patients and sampling for electron microscopy

Case 1

A 28 year old woman was admitted to a hospital on June 18, 1990, in Seoul, Republic of Korea. She had stayed a couple of days in the field prior to this illness. The patient presented with fever, chills, skin rash and 3+ proteinuria at the time of admission. The second day after admission, she started having abdominal cramps, vomiting and diarrhea. General surgery was consulted as she suddenly developed hypotension and hypothermia. The surgical finding was acute haemorrhagic pancreatitis. Her antibody titres against Hantaan virus were 1:12,800 (IgM) and 1:620 (IgG) by ELISA. Her condition deteriorated after development of hypotensive shock. The patient died of irreversible shock with the underlying diagnosis of acute phase HFRS.

Autopsy and sampling were performed 2 days after death. Kidney samples were preserved in a -60°C freezer until processing for electron microscopy. Hantaan virus was isolated from blood of the patient collected 3 days after onset of illness in Vero E6 cell culture.

Case 2

A 30 year old man was admitted to a local hospital in Xian, China in November 11, 1989. He was diagnosed as being in the oliguric phase of acute HFRS complicated with renal failure and shock. IFA specific IgM and IgG antibody titers in serum against Hantaan virus were >40 and >80, respectively. The patient died four days after admission due to acute renal failure and intracranial bleeding.

Renal biopsies were performed 2 hrs after death. A sample of renal tissue approximately 1x3 mm was obtained and preserved at -70°C for use in electron microscopy.

Electron microscopy

All standard procedures of eletron microscopy were used in the two cases. The renal samples were sliced into smaller pieces with a blade, prefixed with periodate-lysine-paraformaldehyde (PLP) for 1 h at 0°C and washed for 30 min with 0.01M phosphate buffer (PBS) at room temperature. For better penetration, some samples were treated with 0.05% Saponin for 40 min, washed with PBS and incubated with an HFRS convalescent serum at 4°C overnight. These were then washed 5 times with PBS at room temperature with gentle shaking, and labelled with protein A-Gold colloid (case 2 only) at a concentration of 1:40 and incubated at 4°C overnight. After extensive washing with PBS (5 times within 5 hrs) at 4°C, each sample was treated with buffered OsO4 for 1 h at 4°C. Dehydration and embedding were processed with graded ethanol and Epon 812, respectively.

Thin sectioning was carried out with a diamond knife (Diatome) on a Sorvall (DuPont) Ultratome. Thin sections were examined on Philips 410 and JOEL-2000 transmission electron microscopes at a magnification of x20,000 to x50,000.

RESULTS

A. Seroepidemiological survey of HFRS and other hemorrhagic diseases among suspect HFRS patients in Korea in 1990 and 1991.

1. Epidemiologic features of HFRS

Total no. of HFRS patients confirmed serologically by Korea University, Seoul Nat'l University and NIH in 1990 and 1991 was 1,043 and 956, respectively as shown in Tables 1 and 2.

There were 537 and 374 hospitalized cases of HFRS confirmed serologically at our Institute in 1990 and 1991. Total no. of serum from suspect HFRS in 1990 and 1991 examined against Hantaan virus and rickettsiae was 3,407 and 2,679 and only 14-16% of them were HFRS patients as shown in Tables 2 and 3. The ratios of serologically confirmed HFRS patients among clinically suspect HFRS patients by civilian doctors and ROK Army are about 13% and 60%, respectively as shown in Table 3. It is noteworthy that ratio of confirmed cases to suspect cases in 1980s is lower than that of 1970s because clinicians sent us sera from only severe cases of suspect HFRS in 1970s while doctors are sending us more sera from mild suspect HFRS patients and other hemorrhagic fever patients in these years. Clinicians have made better clinical diagnosis of HFRS during the epidemic season, October-December, than nonepidemic season of HFRS as shown in Table 4. Patients occured throughout the year and there were two peaks, a small peak in April-July, and a large peak in October-January. One of the new epidemiologic features of HFRS

Table 1.
Hospitalized cases of Hemorrhagic fever with renal syndrome patients in the Republic of Korea.

Year	Korean civilian	Korean soldiers	US soldiers	Total
1951	...	26	827	853
1952	...	18	833	851
1953	...	...	455	455
1954	19	...	307	326
1955	...	...	20	20
1956	...	26	28	54
1957	...	21	13	34
1958	...	20	15	35
1959	...	47	79	126
1960	...	185	10	195
1961	...	341	27	368
1962	...	311	29	340
1963	...	257	11	268
1964	18	205	22	245
1965	2	110	99	211
1966	11	82	36	129
1967	13	86	31	130
1968	26	102	28	156
1969	48	134	9	191
1970	131	221	13	365
1971	391	358	2	751
1972	186	203	0	389
1973	241	237	0	478
1974	176	251	0	427
1975	466	370	1	837
1976	585	304	4	893
1977	288	241	7	536
1978	207	168	10	385
1979	241	122	1	364
1980	185	72	1	258
1981	377	164	2	543
1982	378	123	3	504
1983	402	98	3	503
1984	568	156	6	730
1985	531	159	7	697
1986	530	166	14	710
1987	533	163	5	701
1988	264	97	6	367
1989	306	104	6	416
1990	964	73	6	1,043
1991	912	44	0	956
Total	8,999	5,865	2,976	17,840

Numbers of patients from 1978 to 1989 are serologically confirmed cases at The Institute for Viral Diseases, Korea University and nos. of patients in 1990 and 1991 are serologically confirmed cases at Korea University, Seoul Nat'l University College of Medicine and NIH, Republic of Korea.

Table 2.
HFRS Patients in Korea, 1990 and 1991.

Year	No. of serologically confirmed HFRS patient/ no. of serum from suspect patient at Institute			Total
	Korea Univ.	Seoul Univ.	NIH	
1990	543/3,407 (16%)	293/2,921 (10%)	207/872 (24%)	1,043/7,200 (15%)
1991	374/2,679 (14%)	432/4,250 (10%)	150/712 (21%)	956/7,641 (13%)

(%): Positive rate among tested sera

Table 3.
Total number of Hemorrhagic fever with renal syndrome (HFRS), murine typhus, scrub typhus, spotted fever group (SFG) rickettsiosis and leptospirosis patients diagnosed serologically among suspect hemorrhagic fever patients in Korea, 1990 and 1991.

Disease	Year	No. of patients/no. of serum tested		
		Civilian	ROK soldier	Total
HFRS	90	464/3,268 (14%)	73/139 (53%)	537/3,407 (16%)
	91	330/2,616 (13%)	44/63 (70%)	374/2,679 (14%)
Murine typhus	90	198/3,268 (6%)	0/139 (0%)	199/3,407 (6%)
	91	149/2,616 (6%)	1/63 (1.5%)	150/2,679 (6%)
Scrub typhus	90	683/3,268 (21%)	2/139 (1%)	685/3,407 (20%)
	91	376/2,616 (14%)	1/63 (1.5%)	377/2,679 (14%)
SFG rickettsiosis	90	49/3,268 (2%)	0/139 (0%)	50/3,407 (2%)
	91	10/2,616 (0.4%)	0/63 (0%)	10/2,679 (0.4%)
Leptospirosis	90	140/3,268 (4%)	34/139 (25%)	174/3,407 (5%)
	91	15/2,616 (0.6%)	0/63 (0%)	15/2,679 (0.6%)
Unknown	90	1,734/3,268 (53%)	30/139 (22%)	1,763/3,407 (52%)
	91	1,736/2,616 (66%)	17/63 (27%)	1,753/2,679 (65%)

Table 4.
Number of serologically confirmed cases of Hemorrhagic fever with renal syndrome patients at The Institute for Viral Diseases, Korea University in Korea, 1990 and 1991.

Month	No. of antibody positive sera against Hantaan virus / no. of tested sera from suspect patients						
	Civilian		ROK Army		US Army	Total	
	1990	1991	1990	1991	1990	1990	1991
1	25/108	35/208	10/13	5/5	2/7	37/128	40/213
2	5/57	10/94	0/0	0/0	0/0	5/57	10/94
3	10/103	4/80	0/1	0/0	0/4	10/108	4/80
4	11/117	11/115	0/0	0/0	0/2	11/119	11/115
5	10/117	21/148	3/6	0/0	0/3	13/126	21/148
6	26/128	26/166	1/4	0/0	1/3	28/135	26/166
7	13/103	26/164	0/0	0/0	0/3	13/106	26/164
8	12/170	7/119	1/5	1/2	0/7	13/182	8/121
9	20/237	7/173	1/2	0/0	0/9	21/248	7/173
10	63/725	48/695	14/62	3/6	2/3	79/790	51/701
11	178/1093	83/451	27/28	15/21	1/8	206/1129	98/472
12	91/310	52/203	16/18	20/29	0/4	107/332	72/232
Total (%)	464/3268 (14%)	330/2616 (13%)	73/139 (53%)	44/63 (70%)	6/53 (11%)	543/3460 (16%)	374/2679 (14%)

Table 5.
Geographical distribution of confirmed civilian cases of Hemorrhagic fever with renal syndrome patients at The Institute for Viral Diseases, Korea University in Korea, 1990 and 1991.

Name of province	Year	Month												Total
		1	2	3	4	5	6	7	8	9	10	11	12	
Seoul city	90	4	1	6	5	3	6	5	4	4	12	16	25	91
	91	12	1	2	6	11	7	9	4	0	10	21	13	96
Kyounggi-do	90	13	2	2	4	4	10	4	4	6	20	61	31	161
	91	10	1	1	3	3	7	8	1	3	17	38	32	124
Kangwon-do	90	0	0	0	0	0	2	1	1	2	1	4	1	12
	91	0	1	0	0	1	2	1	1	0	3	6	0	15
Chungcheongbuk-do	90	0	0	1	0	0	0	1	0	1	0	3	2	8
	91	0	1	0	0	2	1	0	0	0	0	5	1	9
Chungcheongnam-do	90	6	1	0	0	0	1	1	1	0	17	35	17	79
	91	9	2	0	0	1	2	6	1	1	10	6	2	39
Kyungsangbuk-do	90	1	0	0	1	0	4	0	1	1	3	9	2	22
	91	0	2	1	0	0	3	0	0	0	2	3	0	11
Kyungsangnam-do	90	0	0	1	0	1	0	0	0	0	1	9	3	15
	91	1	1	0	0	1	2	1	0	1	0	0	0	7
Jeollabuk-do	90	1	0	0	0	0	1	0	0	0	1	4	1	8
	91	2	0	0	0	0	1	1	0	1	4	4	2	17
Jeollanam-do	90	0	0	0	0	1	1	1	1	5	4	32	5	50
	91	1	0	0	0	1	1	0	0	0	1	0	2	6
Jeju-do	90	0	0	0	0	0	0	0	0	0	0	0	0	0
	91	0	0	0	0	0	0	0	0	0	0	0	0	0
Unknown	90	0	1	0	1	1	1	0	0	1	4	5	4	18
	91	0	1	0	2	1	0	0	0	1	1	0	0	6
Total	90	25	5	10	11	10	26	13	12	20	63	178	91	464
	91	35	10	4	11	21	26	26	7	7	48	83	52	330

Table 6.
Occurrence of HFRS patients in districts of Seoul city in 1990 and 1991.

Name of district	No. of patients 1990	1991	Name of district	No. of patients 1990	1991
Yongsan-ku	3	3	Joong-Ku	0	4
Seongbuk-ku	1	3	Jungryang-ku	1	2
Seongdong-ku	7	3	Kwanak-ku	1	5
Yeongdeungpo-ku	1	4	Songpa-ku	4	3
Dobong-ku	16	7	Eunpyung-ku	4	3
Dongdaemun-ku	5	5	Kangseo-ku	3	4
Kuro-ku	10	15	Mapo-ku	3	5
Chongro-ku	2	4	Seocho-ku	3	7
Dongzak-ku	5	4	Seodaemun-ku	5	1
Kangdong-ku	7	4	*Kangnam*-ku	5	3
Nowon-Ku	2	1	Yangcheon-ku	3	6
Total			1990	91	
			1991	96	

Table 7.
Age and sex distribution of HFRS, murine typhus, scrub typhus, spotted fever and leptospirosis among civilian patients in Korea, 1990.

Age	HFRS			murine typhus			scrub typhus			spotted fever			leptospirosis		
	M	F	total	M	F	total	M	F	total	M	F	total	M	F	total
0-10	3	2	5	0	0	0	8	6	14	0	0	0	0	0	0
11-20	18	3	21	3	2	5	11	3	14	1	1	2	1	0	1
21-30	58	13	71	12	9	21	16	29	45	3	2	5	2	0	2
31-40	86	31	117	16	24	40	41	42	83	4	3	7	14	4	18
41-50	47	23	70	27	10	37	60	50	110	8	4	12	19	9	28
51-60	51	40	91	27	14	41	68	75	143	4	3	7	28	11	39
61-70	20	25	45	15	14	29	45	87	132	8	2	10	15	7	22
71-80	5	7	12	2	3	5	22	37	59	0	0	0	9	3	12
81-90	0	0	0	0	1	1	0	4	4	0	1	1	0	0	0
unknown	24	8	32	13	6	19	45	34	79	3	2	5	10	8	18
Total	312 (67%)	152 (33%)	464 (100%)	115 (58%)	83 (42%)	198 (100%)	316 (46%)	367 (54%)	683 (100%)	31 (63%)	18 (37%)	49 (100%)	98 (70%)	42 (30%)	140 (100%)

M : Male F: Female

Table 8.
Age and sex distribution of HFRS, murine typhus, scrub typhus, spotted fever and leptospirosis among civilian patients in Korea, 1991.

Age	HFRS			murine typhus			scrub typhus			spotted fever			leptospirosis		
	M	F	total	M	F	total	M	F	total	M	F	total	M	F	total
0-10	0	0	0	1	0	1	3	3	6	0	0	0	0	0	0
11-20	19	6	25	2	1	3	1	1	2	0	0	0	2	0	2
21-30	49	12	61	9	10	19	21	17	38	1	2	3	1	0	1
31-40	60	21	81	14	8	22	33	20	53	4	0	4	1	1	2
41-50	38	12	50	20	7	27	41	32	73	1	0	1	2	0	2
51-60	34	33	67	19	20	39	37	47	84	0	0	0	4	1	5
61-70	14	15	29	17	7	24	25	45	70	0	0	0	3	0	3
71-80	4	2	6	9	3	12	10	22	32	0	0	0	0	0	0
81-90	0	0	0	0	0	0	1	3	4	0	0	0	0	0	0
unknown	9	2	12	1	1	2	12	2	14	2	0	2	0	0	0
Total	227 (69%)	103 (31%)	330 (100%)	92 (62%)	57 (38%)	149 (100%)	184 (49%)	192 (51%)	376 (100%)	8 (80%)	2 (20%)	10 (100%)	13 (87%)	2 (13%)	15 (100%)

M : Male F: Female

in Korea is the increasing number of HFRS patients in urban areas of Seoul city as shown in Tables 5 and 6. There were 91 and 96 cases of HFRS in Seoul in 1990 and 1991. These patients were only hospitalized severe cases, and usually moderate and mild cases are not included because Seoul virus infection in urban areas is mild and usually diagnosed clinically as influenza or unknown fever. HFRS cases occurred in all districts of Seoul as shown in Table 6 and more patients occured in Dobong-ku and Kuro-ku. Male patients are the dominant group of HFRS as shown in Tables 7 and 8 and the ratio of male to female is about 2:1. Table 9 shows the geographical distribution of HFRS among civilians and about 80% of the patients were in Seoul, Kyunggido, Chungcheongnamdo, and Kangwondo, northern parts of South Korea. Almost all HFRS patients among Korean soldiers occurred in Kyunggido and Kangwondo where main forces of Korean Army is stationed as shown in Table 10.

2. Outbreaks of acute febrile hemorrhagic diseases during the epidemic season of HFRS in 1990 and 1991

As shown in Table 11, the no. of confirmed cases of civilian scrub typhus was 683 and 376 among 3,268 and 2,616 suspect HFRS in 1990 and 1991. These sera from the hospitalized patients were sent to our laboratory from hospitals in and nearby Seoul city for serologic diagnosis of HFRS. The no. of scrub typhus patients among ROK Army is 2 and 1 as shown in Table 14, respectively. Most of murine typhus and scrub typhus patients occurred in October and November, about a month before the large epidemic season of HFRS as shown in Table 11. Geographical distribution of scrub typhus patients in South Korea is shown in Table 9, and most of the patients occurred in Jeolla-do, Seoul city, Kyunggi-do and Chungcheongnam-do and patients also occurred in other provinces as well. About 51%-54% of scrub typhus patients among civilians were female and about 90% of the patients were in the age group of over 21 as shown in Table 8. A large outbreak of murine typhus was demonstrated in 1990 and 1991 as shown in Tables 7-13. It is noteworthy that murine typhus occurred in every month of the year and most of the patients were distributed in Seoul, Kyunggi-do, Chungcheongnam-do, Jeolla-do and Kyungsangnam-do. Of 347 murine typhus patients, 207 were male and 140 were female as shown in Tables 7 and 8. A small outbreak of spotted fever group of rickettsiosis was also demonstrated in summer of 1990 as shown in Table 8. 140 and 15 cases of leptospirosis were diagnosed serologically in 1990 and 1991, respectively as shown in Table 11. Many cases of leptospirosis were found in Jeollanamdo and Kyunggi-do in 1990 and about 80% of them were male.

Table 9.
Geographical distribution of HFRS, murine typhus, scrub typhus, spotted fever group (SFG) rickettsiosis and leptospirosis among suspect civilian hemorrhagic fever patients in Korea, 1990 and 1991.

Name of province	No. of case									
	HFRS		murine typhus		scrub typhus		spotted fever		lepto-spirosis	
	90	91	90	91	90	91	90	91	90	91
Seoul city	91	96	85	65	108	100	21	4	12	9
Kyunggi-do	161	124	31	30	69	76	14	0	32	4
Kangwon-do	12	15	7	4	15	18	0	0	5	1
Chungcheongbuk-do	8	9	5	6	18	11	2	0	5	0
Chungcheongnam-do	79	39	21	22	72	65	2	4	8	0
Kyungsangbuk-do	22	11	3	6	25	8	0	0	2	0
Kyungsangnam-do	15	7	13	4	110	16	3	0	7	0
Jeollabuk-do	8	17	2	6	20	43	2	0	4	0
Jeollanam-do	50	6	23	3	193	26	4	0	50	0
Jeju-do	0	0	2	0	4	0	0	0	0	0
Unknown	18	6	6	3	49	13	1	2	15	1
Total	464	330	198	149	683	376	49	10	140	15

Table 10.
Geographical distribution of HFRS patients among ROK soldiers in Korea in 1990.

Name of area	No. of patient	Name of area	No. of patient
Seoul city	2	Kangwondo	
Kyunggido		Whacheon	3
Paju	7	Chulwon	15
Yeoncheon	13	Samcheok	2
Pocheon	6	Yangku	1
Koyang	4	Inje	2
Yangju	2	Koseong	1
Yangpyung	1	Hongcheon	1
Kimpo	2	Kyungsangdo	
Incheon	1	Andong	1
Fujeongbu	2	Jeollado	
Woongjin	1	Jeonju	1
Chungcheongdo		Unknown	3
Kyesan	1		
Sintan	1		
Total: 73 patients			

Table 11.
Monthly incidence of HFRS, murine typhus, scrub typhus, spotted fever group (SFG) rickettsiosis and leptospirosis among civilian suspect hemorrhagic fever patients in Korea, 1990 and 1991.

Month	Year	HFRS	No. of patient/no. of serum tested				
			murine typhus	scrub typhus	spotted fever	lepto-spirosis	unknown
1	90	25/108	15/108	0/108	7/108	0/108	61/108
	91	35/208	14/208	10/208	3/208	0/208	146/208
2	90	5/57	10/57	2/57	8/57	0/57	32/57
	91	10/94	5/94	1/94	1/94	0/94	77/94
3	90	10/103	29/103	3/103	0/103	1/103	60/103
	91	4/80	0/80	0/80	0/80	0/80	76/80
4	90	11/117	13/117	1/117	0/117	1/117	91/117
	91	11/115	7/115	1/115	2/115	0/115	94/115
5	90	10/117	9/117	1/117	0/117	1/117	96/117
	91	21/148	9/148	0/148	0/148	1/148	117/148
6	90	26/128	6/128	14/128	6/128	0/128	76/128
	91	26/166	4/166	0/166	0/166	4/166	132/166
7	90	13/103	4/103	12/103	15/103	3/103	56/103
	91	26/164	10/164	1/164	2/164	3/164	122/164
8	90	12/170	8/170	31/170	2/170	11/170	164/170
	91	7/119	7/119	0/119	1/119	1/119	103/119
9	90	20/237	14/237	3/237	6/237	35/237	159/237
	91	7/173	19/173	6/173	0/173	3/173	138/173
10	90	63/725	40/725	171/725	1/725	72/725	378/725
	91	48/695	46/695	242/695	1/695	3/695	355/695
11	90	178/109	33/1093	406/1093	1/1093	16/1093	459/1093
	91	83/451	16/451	108/451	0/451	0/451	253/451
12	90	91/310	17/310	39/310	3/310	0/310	160/310
	91	52/203	12/203	7/203	0/203	0/203	123/203
Total	90	464/3268 (14%)	198/3268 (6%)	683/3268 (21%)	49/3268 (2%)	140/3268 (4%)	1734/3268 (53%)
	91	330/2616 (13%)	149/2616 (6%)	376/2616 (14%)	10/2616 (0.4%)	15/2616 (0.6%)	1736/2616 (66%)

Table 12.
Monthly distribution of HFRS, murine typhus, scrub typhus, spotted fever group (SFG) and leptospirosis among suspect civilian hemorrhagic fever patients by sex in Korea, 1990.

Month	HFRS			murine typhus			scrub typhus			spotted fever			leptospirosis		
	M	F	total	M	F	total	M	F	total	M	F	total	M	F	total
1	17	8	25	9	6	15	0	0	0	7	0	7	0	0	0
2	6	2	5	6	4	10	2	0	2	4	4	8	0	0	0
3	5	5	10	19	10	29	0	3	3	0	0	0	1	0	1
4	9	2	11	6	7	13	0	1	1	0	0	0	1	0	1
5	9	1	10	5	4	9	0	1	1	0	0	0	0	1	1
6	21	5	26	4	2	6	6	8	14	3	3	6	0	0	0
7	10	3	13	3	1	4	10	2	12	7	8	15	0	3	3
8	7	5	12	5	3	8	22	9	31	1	1	2	11	0	11
9	14	6	20	10	4	14	3	0	3	5	1	6	22	13	35
10	41	22	63	20	20	40	60	111	171	1	0	1	53	19	72
11	109	69	178	17	16	33	190	216	406	1	0	1	10	6	16
12	67	24	91	11	6	17	23	16	39	2	1	3	0	0	0
Total	312 (67%)	152 (33%)	464 (100%)	115 (58%)	83 (42%)	198 (100%)	316 (46%)	367 (54%)	683 (100%)	31 (63%)	18 (37%)	49 (100%)	98 (70%)	42 (30%)	140 (100%)

M : Male F: Female

Table 13.
Monthly distribution of HFRS, murine typhus, scrub typhus, spotted fever group (SFG) and leptospirosis among suspect civilian hemorrhagic fever patients by sex in Korea, 1991.

Month	HFRS			murine typhus			scrub typhus			spotted fever			leptospirosis		
	M	F	total	M	F	total	M	F	total	M	F	total	M	F	total
1	25	10	35	7	7	14	6	4	10	2	1	3	0	0	0
2	4	6	10	3	2	5	1	0	1	1	0	1	0	0	0
3	4	0	4	0	0	0	0	0	0	0	0	0	0	0	0
4	8	3	11	3	4	7	0	1	1	2	0	2	0	0	0
5	10	11	21	7	2	9	0	0	0	0	0	0	1	0	1
6	17	9	26	3	1	4	0	0	0	0	0	0	3	1	4
7	22	4	26	7	3	10	0	1	1	2	0	2	2	1	3
8	6	1	7	5	2	7	0	0	0	1	0	1	1	0	1
9	3	4	7	12	7	19	2	4	6	0	0	0	3	0	3
10	29	19	48	30	16	46	125	117	242	0	1	1	3	0	3
11	60	23	83	10	6	16	48	60	108	0	0	0	0	0	0
12	39	13	52	5	7	12	2	5	7	0	0	0	0	0	0
Total	227 (69%)	103 (31%)	330 (100%)	92 (62%)	57 (38%)	149 (100%)	184 (49%)	192 (51%)	376 (100%)	8 (80%)	2 (20%)	10 (100%)	13 (87%)	2 (13%)	15 (100%)

M : Male F: Female

Table 14.
Number of HFRS, scrub typhus, murine typhus and spotted fever diagnosed serologically among suspect HFRS patients in ROK soldiers in Korea at The Institute for Viral Diseases, Korea University in 1990 and 1991.

Year		1990		1991	
Total no. of HFRS		73		44	
Total no. of serum tested	=	139	(53%)	63	(70%)
Total no. of scrub typhus		2		1	
Total no. of serum tested	=	139	(1%)	63	(1.5%)
Total no. of murine typhus		0		1	
Total no. of serum tested	=	139	(0%)	63	(1.5%)
Total no. of spotted fever		0		0	
Total no. of serum tested	=	139	(0%)	63	(0%)
Total no. of leptospirosis		34		0	
Total no. of serum tested	=	139	(25%)	63	(0%)
Total no. of unknown sera		30		17	
Total no. of serum tested	=	139	(22%)	63	(27%)

Table 15.
Monthly incidence of HFRS, murine typhus, scrub typhus, spotted fever group (SFG) rickettsiosis and leptospirosis among suspect HFRS in ROK soldiers in Korea at The Institute for Viral Diseases, Korea Uiversity in 1990 and 1991.

Month	HFRS		murine typhus		scrub typhus		spotted fever		lepto-spirosis	
	1990	1991	1990	1991	1990	1991	1990	1991	1990	1991
1	10/13	5/5	0/13	0/5	0/13	0/5	0/13	0/5	0/13	0/5
2	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
3	0/1	0/0	0/1	0/0	0/1	0/0	0/1	0/0	0/1	0/0
4	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
5	3/6	0/0	0/6	0/0	2/6	0/0	0/6	0/0	0/6	0/0
6	1/4	0/0	0/4	0/0	0/4	0/0	0/4	0/0	0/4	0/0
7	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
8	1/5	1/2	0/5	0/2	0/5	0/2	0/5	0/2	0/5	0/2
9	1/2	0/0	0/2	0/0	0/2	0/0	0/2	0/0	0/2	0/0
10	14/62	3/6	0/62	0/6	0/62	0/6	0/62	0/6	34/62	0/6
11	27/28	15/21	0/28	0/21	0/28	1/21	0/28	0/21	0/28	0/21
12	16/18	20/29	0/18	1/29	0/18	0/29	0/18	0/29	0/18	0/29
Total	73/139 (53%)	44/63 (70%)	0/139 (0%)	1/63 (1.5%)	2/139 (1%)	1/63 (1.5%)	0/139 (0%)	0/63 (0%)	34/139 (25%)	0/63 (0%)

Table 16.
Number of HFRS, scrub typhus, murine typhus and spotted fever diagnosed serologically among suspect HFRS patients in US Army soldiers in Korea at The Institute for Viral Diseases, Korea University in 1990.

Total no. of HFRS		1	
Total no. of serum tested	=	2	(50 %)
Total no. of scrub typhus		0	
Total no. of serum tested	=	2	(0 %)
Total no. of murine typhus		0	
Total no. of serum tested	=	2	(0 %)
Total no. of spotted fever		1	
Total no. of serum tested	=	2	(50 %)
Total no. of leptospirosis		0	
Total no. of serum tested	=	2	(0 %)
Total no. of unknown sera		0	
Total no. of serum tested	=	2	(0 %)

Table 17.
Monthly incidence of HFRS, murine typhus, scrub typhus, spotted fever group (SFG) rickettsiosis and leptospirosis among suspect US Army soldiers in korea at The Institute for Viral Diseases, Korea Uiversity in 1990.

Month	HFRS	murine typhus	scrub typhus	spotted fever	lepto-spirosis
1	0/0	0/0	0/0	0/0	0/0
2	0/0	0/0	0/0	0/0	0/0
3	0/0	0/0	0/0	0/0	0/0
4	0/0	0/0	0/0	0/0	0/0
5	0/0	0/0	0/0	0/0	0/0
6	1/2	0/0	0/0	1/2	0/0
7	0/0	0/0	0/0	0/0	0/0
8	0/0	0/0	0/0	0/0	0/0
9	0/0	0/0	0/0	0/0	0/0
10	0/0	0/0	0/0	0/0	0/0
11	0/0	0/0	0/0	0/0	0/0
12	0/0	0/0	0/0	0/0	0/0
Total	1/2 (50%)	0/0 (0%)	0/0 (0%)	1/2 (50%)	0/0 (0%)

In the Korean Army, HFRS is a major military problem and leptospirosis is next as shown in Table 14. In the U.S. Army in Korea, HFRS and spotted fever were confirmed serologically in summer of 1990 as shown in Tables 16 and 17.

B. Seroepidemiologic survey of hantavirus infection among wild bats and wild birds in Korea

1. Serologic evidence for hantavirus infection in wild bats

Total no. of captured wild bats in the endemic areas of HFRS in 1990 was 143 and only 2 species of bats were identified. Seropositive bats were 6, 5 positive Rhinolophus ferrum-equinum and 1 positive Vespertilo abramus, as shown in Table 18. Antibody titers against Hantaan and Seoul viruses ranged 16-256, and none of the bats were positive against Puumala vius as shown in Table 19. In this limitted study, seropositive bats to Hantaan and Seoul viruses were captured only in Kyunggi and Chungnam Provinces as shown in Table 20. Attempts to isolate hantavirus from lungs of seropositive rats in Vero E6 cell culture were failed.

2. Serologic evidence for hantavirus infection in wild birds

As shown in Table 21, 166 wild birds of 15 species, 8 species of local birds and 7 species of migratory birds, were captured in 1989-1990 and only 2 species of local birds, Paradoxomis webbiana and Emberiza elegans, were antibody positive against Hantaan virus. Antibody titers of 15 wild birds were 16-256 as shown in Table 22. Antibody positive birds against Hantaan virus were captured in Kyunggi-do Yangju-kun and Yeonchon-kun in October-December 1990, hyperendemic areas of HFRS since 1951 as shown in Table 23. Total no. of wild birds captured in the endemic areas of HFRS in 1991 is 268 and 30 of the birds were antibody positive to Hantaan virus and 1 was positive to Seoul virus as shown in Tables 24 and 25. Antibody positive species of the birds were Paradoxomis webbiana, Emberiza rustica and Parus major. Most of the positive birds were Paradoxomis webbiana captured in October-December 1991 during epidemic season of HFRS in Kyunggi-do. It is clear that the local wild birds are infected with Hantaan virus and these wild birds may play an important role as reservoir of Hantaan virus in Korea.

C. Puumala coated high density particle (HDP) agglutination against hantavirus antibodies

A simple serologic diagnostic test with Puumala virus coated HDP was developed by modified method of Hantaan HDP agglutination as previously described (42). The optimum antigen coating concentration was four to eight ELISA units/ml, while negative reactions were found with diluent. Alternatively, non-coated HDP antigen controls were always negative against both diluent and serum containing anti-

Table 18.
Distribution of Hantaviral IF antibodies in the wild bats caught in Korea, 1990.

Species name	Immunofluorescent antibody against		
	HTNV	SEOV	PUUV
Rhinolophus ferrum-equinum	3/122 (2.4%)	2/122 (1.6%)	0/122
Vespertilo abramus	1/ 21 (4.8%)	0/ 21	0/ 21
Total	4/143 (2.8%)	2/143 (1.4%)	0/143

HTNV : Hantaan virus SEOV : Seoul virus PUUV : Puumala virus

Table 19.
IF antibody titers of positive wild bats against Hantaan, Seoul and Puumala viruses.

Code no. of bird	Species name	IF antibody titers against		
		HTNV	SEOV	PUUV
Bat/90-1	Rhinolophus ferrum-equinum	256	-	-
Bat/90-17	Vespertilo abramus	32	-	-
Bat/90-27	Rhinolophus ferrum-equinum	-	16	-
Bat/90-32	Rhinolophus ferrum-equinum	-	16	-
Bat/90-43	Rhinolophus ferrum-equinum	64	-	-
Bat/90-49	Rhinolophus ferrum-equinum	16	-	-

HTNV : Hantaan virus SEOV : Seoul virus PUUV : Puumala virus

Table 20.
Serologic survey of the wild bats for Hantavirus antibodies in Korea, 1990.

Area	Date of collection	Species name	No. of antibody positive/no. of serum tested against		
			HTNV	SEOV	PUUV
Kyunggi	Feb. 1990	Rhinolophus ferrum-equinum	1/1	0/1	0/1
Chungbuk Danyang-kun	Feb. 1990	Vespertilo abramus	0/4	0/4	0/4
Kangwon Yeongwal-kun	Feb. 1990	Vespertilo abramus	0/2	0/2	0/2
Chungnam Cheongwon-kun	Feb. 1990	Vespertilo abramus	0/8	0/8	0/8
Chungnam Chunan-kun	Feb. 1990	Rhinolophus ferrum-equinum	0/3	0/3	0/3
		Vespertilo abramus	1/4	0/4	0/4
Chungnam Kongju-kun	Feb. 1990	Rhinolophus ferrum-equinum	2/56	2/56	0/56
Chungnam Chunwon-kun	Feb. 1990	Rhinolophus ferrum-equinum	0/7	0/7	0/7
Kyungbuk Chungsong-kun	Jun. 1990	Rhinolophus ferrum-equinum	0/3	0/3	0/3
Chungnam Kongju-kun	Jul. 1990	Rhinolophus ferrum-equinum	0/6	0/6	0/6
		Vespertilo abramus	0/2	0/2	0/2
Kangwon Pyungchang-kun	Jul. 1990	Rhinolophus ferrum-equinum	0/9	0/9	0/9
Kyungbuk Uljin-kun	Aug. 1990	Rhinolophus ferrum-equinum	0/3	0/33	0/3
		Vespertilo abramus	0/1	0/1	0/1
Kyungbuk Kyungju-kun	Aug. 1990	Rhinolophus ferrum-equinum	0/32	0/32	0/32
		Total	4/141	2/141	0/141

HTNV : Hantaan virus SEOV : Seoul virus PUUV : Puumala virus

Table 21.
Distribution of Hantaviral IF antibodies in wild birds caught in Korea, 1989-1990.

Species name	No. of antibody positive/ no. of serum tested against virus		
	HTNV	SEOV	PUUV
Paradoxomis webbiana	14/55 (26%)	0/55	0/55
Emberiza elegans	1/47 (2%)	0/47	0/47
Passer montanus	0/35	0/35	0/35
Parus major	0/11	0/11	0/11
Phoenicurus auroreus	0/3	0/3	0/3
Streptopelia orientalis	0/3	0/3	0/3
Lanis tigrinus	0/2	0/2	0/2
Eophona migratoria	0/2	0/2	0/2
Lanis bucephalus	0/2	0/2	0/2
Emberiza rustica	0/1	0/1	0/1
Pica pica	0/1	0/1	0/1
Eurystomus orientalis	0/1	0/1	0/1
Accipiter soloensis	0/1	0/1	0/1
Upupa epops	0/1	0/1	0/1
Motacilla alba	0/1	0/1	0/1
Total	15/166	0/166	0/166

HTNV : Hantaan virus SEOV : Seoul virus PUUV : Puumala virus

Table 22.
IF antibody titers of positive wild birds against Hantaan, Seoul and Puumala viruses.

Code no. of bird	Species name	IF antibody titers against		
		HTNV	SEOV	PUUV
Bird/90-7	Paradoxomis webbiana	32	-	-
Bird/90-8	Paradoxomis webbiana	16	-	-
Bird/90-15	Emberiza elegans	16	-	-
Bird/90-17	Paradoxomis webbiana	16	-	-
Bird/90-27	Paradoxomis webbiana	16	-	-
Bird/90-32	Paradoxomis webbiana	32	-	-
Bird/90-43	Paradoxomis webbiana	256	-	-
Bird/90-49	Paradoxomis webbiana	16	-	-
Bird/90-51	Paradoxomis webbiana	16	-	-
Bird/90-54	Paradoxomis webbiana	16	-	-
Bird/90-56	Paradoxomis webbiana	16	-	-
Bird/90-57	Paradoxomis webbiana	16	-	-
Bird/90-58	Paradoxomis webbiana	16	-	-
Bird/90-59	Paradoxomis webbiana	16	-	-
Bird/90-62	Paradoxomis webbiana	16	-	-

HTNV : Hantaan virus SEOV : Seoul virus PUUV : Puumala virus

Table 23.
Serologic survey of the wild birds for Hantavirus antibodies in Korea, 1989-1990.

Area	Date of collection	Species name	No. of antibody positive/no. of serum tested against virus		
			HTNV	SEOV	PUUV
Kyunggi-do Gapyung-kun	Jun. 1989	Passer montanus	0/31	0/31	0/31
	"	Lanius tigrinus	0/2	0/2	0/2
	"	Eophona migratoria	0/2	0/2	0/2
	"	Paradoxomis webbiana	0/1	0/1	0/1
	"	Motacilla alba	0/1	0/1	0/1
	"	Upupa epops	0/1	0/1	0/1
	"	Eurystomus orientalis	0/1	0/1	0/1
	"	Accipiter soloensis	0/1	0/1	0/1
Kyunggi-do Pyungtek-kun	Nov. 1989	Streptopelia orientalis	0/1	0/1	0/1
Kyunggi-do Pyungtek-kun	Dec. 1989	Emberiza elegans	0/43	0/43	0/43
	"	Parus major	0/10	0/10	0/10
	"	Phoenicurus auroreus	0/3	0/3	0/3
	"	Lanius bucephalus	0/2	0/2	0/2
	"	Pica pica	0/1	0/1	0/1
	"	Emberiza rustica	0/1	0/1	0/1
	"	Passer montanus	0/1	0/1	0/1
Chungnam Daeduck-kun	Oct. 1990	Passer montanus	0/1	0/1	0/1
Kyunggi-do Yangju-kun	Oct. 1990	Paradoxomis webbiana	2/8	0/8	0/8
		Passer montanus	0/2	0/2	0/2
Kyunggi-do Yeonchon-kun	Nov. 1990	Emberiza elegans	1/4	0/4	0/4
		Parus major	0/1	0/1	0/1
Kyunggi-do Yangju-kun	Dec. 1990	Paradoxomis webbiana	5/33	0/33	0/33
Kyunggi-do Yangju-kun	Dec. 1990	Paradoxomis webbiana	7/13	0/13	0/13
		Total	15/166	0/166	0/166

HTNV : Hantaan virus SEOV : Seoul virus PUUV : Puumala virus

Table 24.
Distribution of Hantaviral IF antibodies in wild birds caught in Korea, 1991.

Species name	No. of antibody positive/ no. of serum tested against virus		
	HTNV	SEOV	PUUV
Paradoxmis webbiana	27/186 (15%)	0/186	0/186
Emberiza rustica	3/51 (6%)	1/51 (2%)	0/51
Emberiza elegans	0/3	0/3	0/3
Troglodytes troglodytes	0/1	0/1	0/1
Passer montanus	0/4	0/4	0/4
Pericrocotus divaricatus	0/1	0/1	0/1
Parus major	1/9 (11%)	0/1	0/1
Montacilla alba	0/1	0/1	0/1
Turdus obscurus	0/4	0/4	0/4
Turdus naumanni	0/5	0/5	0/5
Phoenicurus auroreus	0/1	0/1	0/1
Coturnix caturnix	0/2	0/2	0/2
Total	31/268	1/268	0/268

HTNV : Hantaan virus SEOV : Seoul virus PUUV : Puumala virus

Table 25.
Serologic survey of the wild birds for Hantavirus antibodies in Korea 1991.

Area	Date of collection	Species name	No. of antibody positive/no. of serum tested against virus		
			HTNV	SEOV	PUUV
Kyunggi-do Yangju--kun	Jan. 1991	Emberiza elegans	0/1	0/1	0/1
	"	Emberiza rustica	0/1	0/1	0/1
	"	Paradoxomis webbiana	1/49	0/49	0/49
	"	Troglodytes troglodytes	0/1	0/1	0/1
Seoul city Seongbuk-ku	Jul. 1991	Passer montanus	0/3	0/3	0/3
	"	Pericrocotus divaricatus	0/1	0/1	0/1
	"	Parus major	0/7	0/7	0/7
Kyunggi-do Yeoncheon-kun	Jul. 1991	Montacilla alba	0/1	0/1	0/1
	"	Turdus obscurus	0/4	0/4	0/4
	"	Paradoxomis webbiana	1/3	0/3	0/3
Kyunggi-do Yeoncheon-kun	Oct. 1991	Emberiza rustica	3/46	1/46	0/46
	"	Paradoxomis webbiana	0/7	0/7	0/7
	"	Parus major	1/1	0/1	0/1
	"	Emberiza elegans	0/2	0/2	0/2
Kyunggi-dp Yeoncheon-kun	Nov. 1991	Paradoxomis webbiana	9/64	0/64	0/64
Kyunggi-do Paju-kun	Dec. 1991	Emberiza rustica	0/4	0/4	0/4
	"	Turdus naumanni	0/5	0/5	0/5
	"	Phoenicurus auroreus	0/1	0/1	0/1
	"	Parus major	0/1	0/1	0/1
	"	Passer montanus	0/1	0/1	0/1
	"	Coturnix coturnix	0/2	0/2	0/2
	"	Paradoxomis webbiana	16/63	0/63	0/63
		Total	31/268	1/268	0/268

HTNV : Hantaan virus SEOV : Seoul virus PUUV : Puumala virus

Table 26.
Comparison of antibody titers of sera from suspect nephropathia epidemica patients against hantavirus by HDPA, ELISA and IFA tests.

Code no. of serum	Antibody titers by					
	IFAT		HDPA		ELISA	
	HTNV	PUUV	HTNV	PUUV	HTNV	PUUV
NE/267-85	512	8192	126	8192	256	8192
NE/769-85	-	-	-	-	-	-
NE/772-85	-	-	-	-	-	-
NE/774-85	512	8192	256	8192	1024	16384
NE/775-85	-	-	-	-	-	-
NE/777-85	32	2048	32	2048	64	2048
NE/786-85	512	8192	64	8192	512	8192
NE/893-85	128	8192	64	4096	128	8192
KHF/814-91	4096	512	8192	512	8192	256
KHF/821-91	8192	1024	8192	512	8192	512
EHF/J-1	2048	512	4096	256	4096	256
US/control	-	-	-	-	-	-

NE : Nephropathia epidemica
KHF : Korean hemorrhagic fever
EHF : Epidemic hemorrhagic fever, Japan

bodies. Comparative antibody titers of sera from HFRS patients in the different parts of the world are shown in Table 26. Puumala virus antigen-coated HDP reacted with not only antibody to Hantaan virus (sera from Korea) but also with antibodies to Seoul virus (sera from Japan) and with antibodies to Puumula virus (sera from Finland). It was found that HDPA titers were about same as IFA titers.

D. Electron microscopy of kidneys of HFRS patients

1. General findings

Several pathological changes were seen in the renal medulla. There was oedema in the interstitium with swelling, necrosis and detachment of the epithelium (Fig. 1 and 2). Some destructive changes were so remarkable that there were almost no cellular structures recognizable. Only some naked basement membranes with necrotic cell debris and clusters of electron dense precipitates underneath were observed (Fig. 2). The lumen of some degenerated renal tubules were often filled with dense homogenous substances and detached necrotic epithelia (Fig. 2). The basement membranes seemed to be widened and disorganized, especially where the electron dense precipitates and Hantaan virion-like structures were found.

2. Hantaan virus-like structures

Hantaan virus-like particles were distinctly visualized in the matrix of the basement membranes (Figs. 4-6), The virus-like particles resembled Hantaan virions (Fig. 7), except for their large variations in size, ranging from 80 nm to 300 nm. Nevertheless, they all shared the fundamental structures of a Hantaan virion, such as: a well delineated envelope (membrane) and a granulated inner component (nucleocapsid). Sometimes, bizarre particles deviating from the normal structure of a Hantaan virion were observed. None of these particles were labelled by immune colloidal gold.

3. Inclusion bodies

Some typical Hantaan virus inclusion bodies, especially those of filamentous form were found in the cytoplasm of the epithelial cells of the renal tubules. At higher magnification, one can clearly see the fibrils and microtubules making up the inclusion bodies (Figs. 8-10).

4. Electron dense precipitates

The electron dense precipitates were frequently seen in the oedematous interstitial area surrounding the basement membrane. Some of the fibrous precipitates in the Saponin treated sections were labelled with gold colloids. The electron dense components of the precipitates were labelled with gold particles.

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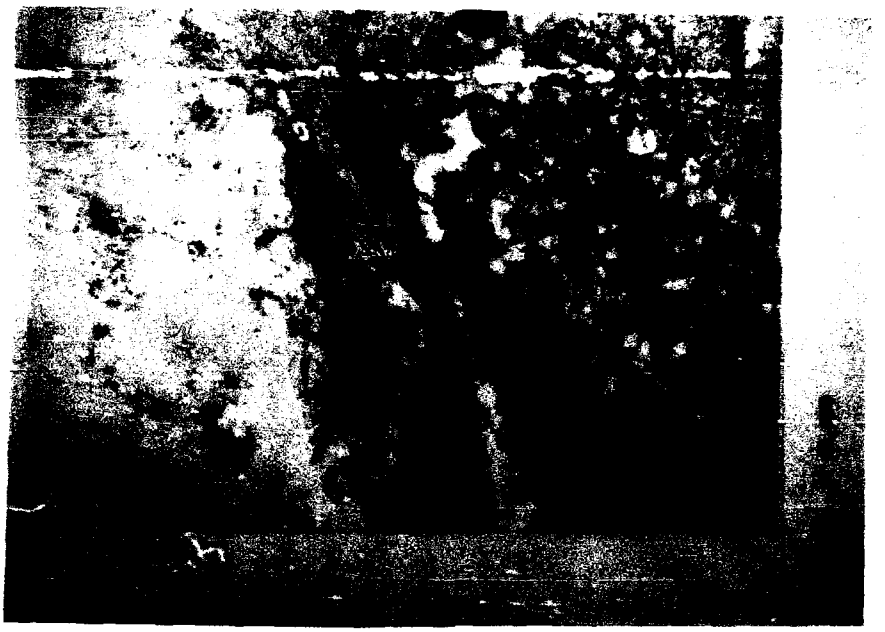

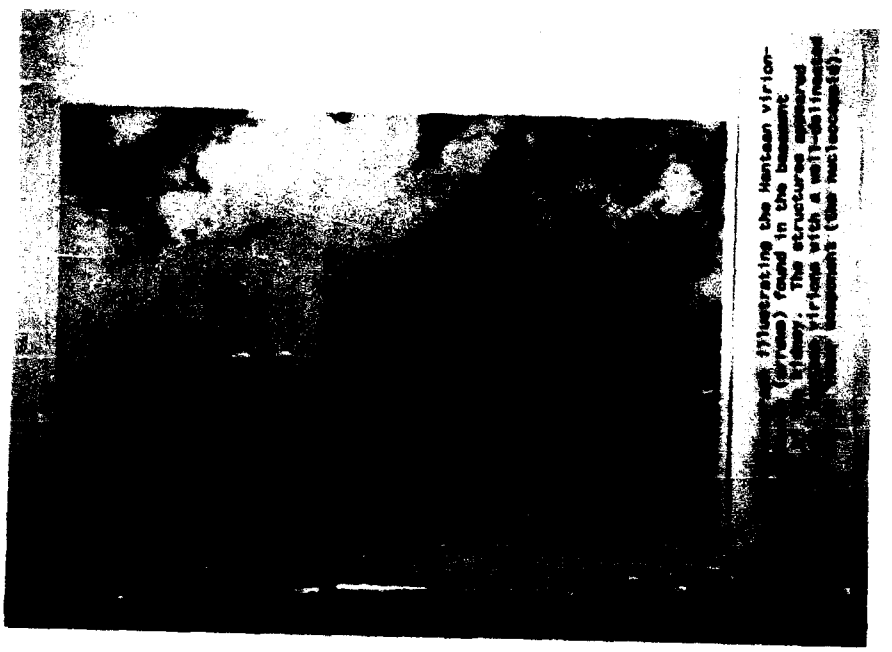

(Figure) found in the basement
kidney. The structures appeared
virions with a multilaminated
component (the nucleocapsid).

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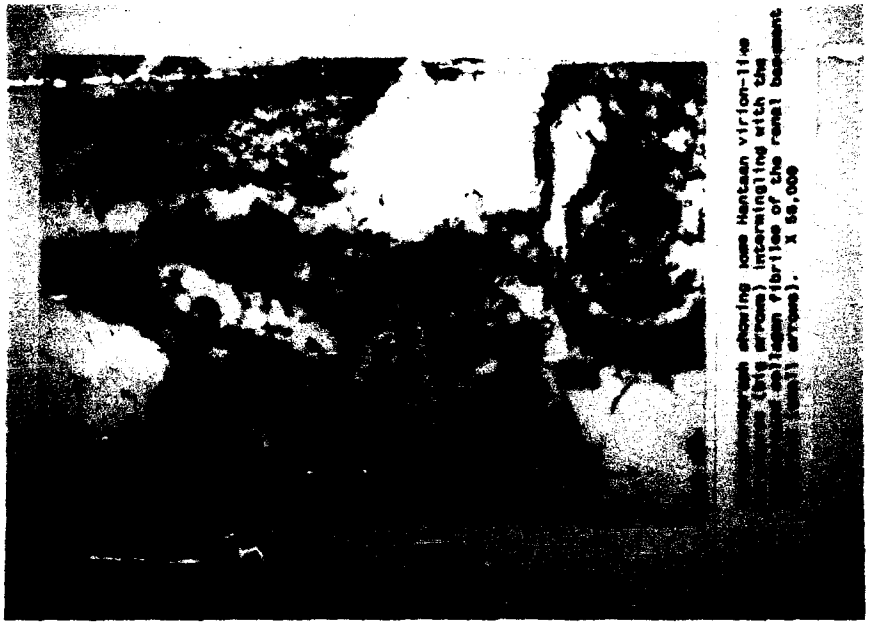

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5. Surface antigen layer-like structure

Some antigen layer-like structures were occasionally seen in the intercellular space of the medulla of the kidneys (Fig. 10). The appearance of the electron dense materials and its intrinsic tortuous shape resembles the viral antigen layer (VAL) seen on the surface of Hantaan virus infected cells (Fig. 8) (43,45).

E. Serologic diagnosis of sera from suspect HFRS against different serotypes of hantavirus by IFAT

There have been claims against serodiagnosis of HFRS from clinicians in Korea because some of the suspected HFRS patients who had typical clinical manifestations and laboratory findings of HFRS were sero-negative against Hantaan virus by IFAT. Therefore, it was decided to screening these sera from suspect HFRS against not only Hantaan virus 76/118 and also Hantaan virus HWL strain, Seoul virus 80/39, and Puumala virus(141247) simultaneously by IFAT.

Table 27 shows antibody titers of sera from 18 suspect HFRS in 1990 against different prototype serotypes of Hantavirus. Five patients out of 18 patients were antibody negative to Hantaan virus 76/118, only 1 patient out of 18 patients was negative to Hantaan-virus HWL, 8 of 18 patients were negative to Seoul virus 80/39 and 11 of 18 patients were negative to Puumala virus. Hantaan virus HWL is better antigen than Hantaan and Seoul virus antigens for serodiagnosis of HFRS. It is noteworthy that one serum from the patient ROK90-65 contained only IF antibody to Puumala virus and a serum from ROK90-84 contained higher anti-Puumala antibody than anti-Hantaan or anti-Seoul antibodies.

Table 28 shows the results of comparative antibody titration of 9 sera from suspect HFRS who were diagnosed as HFRS clinically in 1991 against 4 serotypes of hantavirus. Five out of 9 sera were negative to Hantaan virus 76/118 antigen, 2 of 9 sera were negative to Hantaan virus HWL antigen, 5 of 9 sera were negative to Seoul virus 80/39 and 3 of 9 sera were negative to Puumala virus antigen. It is clear that Hantaan virus HWL strain did show better results than Hantaan virus 76/118 strain and 2 sera from the patients 91-672 and 91-688 were only positive against Puumala virus. This is the first results that some sera from suspect HFRS in Korea were only positive to Puumala virus.

Table 29 shows the PRN antibody titers of 8 sera from HFRS patients who had high IF antibody titers to Puumala virus than Hantaan and Seoul viruses and one serum KHF91-2351-2 that contained high antibody titers to Hantaan HWL than Hantaan 76/118. It was strange that none of 8 sera that had high IF antibody titers to Puumala virus contained PRN antibodies to Puumala virus but 3 sera (ROK90-65-1, KHF91-367, KHF91-688-1) rather contained relatively high PRN antibodies to Hantaan virus 76/118. It is difficult to conclude that what virus was the causative agent of these patients.

Table 27.
Comparative antibody titers of sera against different serotypes of Hantavirus from HFRS patients in Korea by IFAT.

Code no. of HFRS sera	HTNV (76/118)	HTNV (HWL)	SEOV (80/39)	PUUV (NE141247)	PHV (#168222)
1. ROK-90-2	512	512	256	32	
2. ROK-90-8-1	32	32	128	-	
ROK-90-8-2	32	32	128	-	
3. ROK-90-9-1	16,384	16,384	8,192	2,048	
ROK-90-9-2	16,384	16,384	8,192	2,048	
4. ROK-90-14	32	128	-	-	-
5. ROK-90-22	-	64	-	-	-
6. ROK-90-28	32	32	64	-	
7. ROK-90-30	32	64	-	-	
8. ROK-90-55	-	32	-	-	
9. ROK-90-58-1	-	64	64	-	
ROK-90-58-2	-	64	64	-	
10. ROK-90-64-1	-	32	-	-	
ROK-90-64-2	-	32	-	-	
11. ROK-90-65-1	-	-	-	128	-
ROK-90-65-2	-	-	-	128	
12. ROK-90-68	64	256	-	-	-
13. ROK-90-78	256	512	-	-	
14. ROK-90-84	32	64	32	256	-
15. ROK-90-97	32	128	32	-	
16. ROK-90-100	8,192	8,192	2,048	128	
17. ROK-90-101	16,384	16,384	8,192	32	
18. ROK-90-116	16,384	16,384	8,192	128	
Total	13/18 (72.2%)	17/18 (94.4%)	10/18 (55.6%)	7/18 (38.9%)	0/5

Table 28.
Comparative screening and antibody titers of sera from HFRS patients in Korea against different serotypes of hantavirus by IFAT.

Code no. of HFRS and control sera	Antibody titers to hantaviruses			
	HTNV (76/118)	HTNV (HWL)	SEOV (80/39)	PUUV (NE141247)
1. HFRS-91-668	256	256	64	128
2. HFRS-91-671	32	256	512	32
3. HFRS-91-672	-	-	-	32
4. HFRS-91-680	128	64	64	32
5. HFRS-91-688	-	-	-	128
6. HFRS-91-696	-	128	-	-
7. HFRS-91-699	128	128	-	-
8. HFRS-91-702	-	64	64	128
9. HFRS-91-712	-	64	-	-
(Control)				
(HTNV) HFRS-89-825	8192	4096	2048	64
(SEOV) Song/JW	1024	512	2048	128
(PUUV) Fin-653	512	8192	8192	16384

HTNV: Hantaan virus SEOV: Seoul virus PUUV: Puumala virus

Table 29.
Comparative IF and PRN antibody titers of sera from HFRS patients (high IF antibody titers to PUUV) in Korea against hantaviruses.

Code no. of HFRS sera	Antibody titers against hantaviruses							
	HTNV (76/118)		HTNV (HWL)	SEOV (80/39)		PUUV (NE141247)		PHV (#168222)
	IF	PRNT	IF	IF	PRNT	IF	PRNT	IF
1. ROK-90-65-1	-	160	-	-	10	128	10	-
2. ROK-90-84	32	10	64	32	<10	256	10	-
3. KHF-91-262	-	40	32	-	<10	256	<10	-
4. KHF-91-367	-	160	-	-	10	64	<10	-
5. KHF-91-420	-	10	-	-	<10	1024	<10	-
6. KHF-91-688-1	-	160	-	-	10	128	<10	-
7. KHF-91-702	-	40	64	64	<10	128	<10	-
8. KHF-91-950-1	-	10	-	-	<10	64	<10	-
9. KHF-91-2351-2	32	10	256	-	<10	-	<10	-

DISCUSSION

It has been known that HFRS occurs in Korea since Korean war, 1951. The 537 and 374 cases of HFRS represent only serologically confirmed hospitalized patients at our Institute in 1990 and 1991. Sera from suspect HFRS patients came from limited hospitals in and nearby city of Seoul, therefore, the real total no. of HFRS in entire South Korea should be at least three times more than no. of patients in table 1 because it is estimated that we might have examined about one third of HFRS cases according to the distribution of population and areas we covered. It could be estimated that there are at least 2,000 cases of HFRS patient in S. Korea every year if serologic diagnostic capabilities are available at hospitals in the endemic areas of S. Korea. In fact, total no. of serologically confirmed cases of HFRS by 3 Institutes in Seoul, Korea University Institute of Viral Diseases, Seoul National University Medical College and NIH, was 1,043 and 956 in 1990 and 1991, respectively. About 90% of total patients were distributed in 4 Provinces located in northern parts of S. Korea as shown in table 5 but it does not mean that the 4 Provinces are more heavily infected foci of HFRS than other Provinces since other Provinces are far from Seoul and it is very difficult to send sera from the local hospitals to Seoul for serologic diagnosis of the disease though there are many patients. Occurence of HFRS patient in Seoul city is in all districts and every district had several cases of HFRS every year. It remains to be studied the risk factors and virulence of Seoul virus strains where about 10 million people are living and more than 10% of urban rats population is infected with Seoul virus (32). There is a large epidemic peak of HFRS in late fall as before and a minor peak in May-July every year (7) and there were 73 and 44 cases among Korean soldiers stationed near DMZ between South and North Korea in 1990 and 1991. There were only 6 HFRS and one died among about 40,000 U.S. soldiers stationed in Korea in 1990. It is noteworthy that no. of HFRS in Korean Army is dramatically decreasing since 1989 and only 44 cases were confirmed in 1991.

It has been known that the clinical complex of acute hemorrhagic diseases occur in summer and fall in Korea since 1982 and these are HFRS, leptospirosis and rickettsioses (37). It is surprising to learn that there were 149-198 cases of murine typhus, 376-683 cases of scrub typhus and 10-49 spotted fever patients in 1990 and 1991.

However, still about 50-65% of total suspect HFRS patients was not diagnosed serologically and remains to be answered and we are trying to find the etiologic agents of these unknown fevers. We have the serologic evidence of existence of Colorado tick fever and RMSF like illness in Korea. Collaborative study to search the causative agents of the unknown fevers among hemorrhagic diseases between USAMRU/Korea, USAMRIID, CDC in Colorado and our Institute is in progress.

It is also planned to isolate local strains of R. typhi and R. siberica from wild rodents and their ectoparasites in the near future.

It has been recognized that HDP sensitizes more proteinous and lipid antigen on their surface, which results in higher sensitivity to antibody than similarly used erythrocytes of polystyrene latex particles (40). As for hantavirus HDPA, we found that this test provides higher sensitivity than IFAT, which may depend on this binding property of HDP (42). The highly purified Puumala virus antigen as here applied had little cross reaction with antisera to Seoul and Hantaan viruses. Accordingly, as lyophilized antigen-coated HDP were used, this reaction is easily used for measurement of Puumala virus antibody, without any technical complexity, within 1 hr. The available serologic diagnostic tests for HFRS are IFAT, ELISA, plaque-reduction neutralization test, hemagglutination inhibition test and an immune adherence hemagglutination test (7), but these tests are complicated and time-consuming compared with Hantaan and Puumala HDPA test.

Since the report of isolation of Hantaan virus from lungs of wild birds captured in Far East region of Russia by Tkachenko et al (46), we were interested in to find the serologic evidence of Hantavirus infection among wild bats and birds living in the endemic areas of HFRS in S. Korea. As expected, 2 species of common bats and 2 species of local birds were infected with Hantaan virus. The results indicate strongly that wild bats and wild birds may play an important role as reservoirs of hantaviruses and spread hantaviruses rapidly from a region to another region, and this might be one of the reasons for ubiquitous prevalence of hantaviruses throughout the world.

In the morphogenesis of Hantaan virus, several antigens were found in the cytoplasm of Vero-E6 cells. The antigens have been identified to be either Hantaan virions themselves or their gene products manifesting in cells in the form of inclusion bodies (granular, filamentous, and/or a mixture of both), viral antigen layer on the cells surface (VAL), or as virion associated granules (43-45). It has previously been postulated that the enormous quantity of viral antigens presented in cells must have something to do with the pathogenesis of HFRS.

The present research was carried out directly on the target organs (kidneys) of acute HFRS cadavers, aiming to explore some clues, if any, on the pathogenesis of Hantaan virus or of Hantaan related antigens.

Electron microscopic study revealed that all Hantaan virus related structures (except VAL), previously described, were found in the kidneys of acute phase HFRS cadavers. This fact indicates that the kidney is indeed the direct target organ of Hantaan virus.

As described previously, the diverse polymorphic appearance of the Hantaan virus is a striking and important morphologic property. Consistent with previous findings, there was a remarkably large size variation among the Hantaan virion-like structures found in the acute phase KHF cadavers. It must be noted, however, that the many different morphologic manifestations of the virus may also be due to postmortem changes of artifacts caused by preparation procedures.

The immune colloidal gold that used in our methods could not penetrate into the tissues. This same technical problem of penetration also occurred with the monolayer cell culture. Additionally, to find an acute phase KHF cadaver is extremely difficult. As such, we were not able to repeat the experiments to more definitively identify some of the more characteristic structures, e.g., inclusion bodies, of the Hantaan virus. This is why we choose to describe the structures we identified, not as definite Hantaan virions, but as Hantaan virus-like structures.

The presence of Hantaan virus related structures in association with severe destructive pathological changes may serve as strong evidence of the direct attack and multiplication of Hantaan virus in kidneys. These pathological cellular changes may be some of the most important factors leading to the lethal outcome of HFRS patients. The immune-gold colloidal labeling revealed the existence of great quantities of electron dense deposits; deposits partly labelled by HFRS convalescent serum. These deposits might represent antigen-antibody complex precipitates at the level of the basement membrane. This may also suggest putatively that an immune mediated process could play some role in the pathogenesis of HFRS. Further studies of cases of acute HFRS will be needed to further define the precise and definite roles of Hantaan virus and Hantaan virion-like structures in the pathogenesis of HFRS.

There have been some claims from the clinicians about negative results of serologic test of suspect HFRS since application of IFAT with Hantaan virus antigen for serologic diagnosis of HFRS in Korea. The cliam is that about 2-3% of suspect HFRS patients who were diagnosed clinically as HFRS were IF antibody negative against Hantaan virus. It was demonstrated that IFAT with Hantaan virus antigen for serologic diagnosis of HFRS and seroepidemiologic survey of Hantavirus infection in man and animal was good becuase IF antibodies against hantaviruses cross react each other and last a long time according to the previous results (7,18-21,28,39).

Recently, we decided to test the sera from suspect HFRS who were negative to Hantaan virus antigen against different strain of Hantaan virus, Seoul virus and Puumala virus by IFAT. As shown in Tables 26 and 27, several patients who

were antibody negative to Hantaan virus 76/118 strain in 1990 and in 1991 were antibody positive to either Hantaan virus HWL strain or Puumala virus. After these findings, we are now screening all sera from suspect HFRS patients against 4 strains of Hantavirus, Hantaan 76/118, Hantaan HWL, Seoul virus 80/39 and Puumala virus #141247, for serodiagnosis of HFRS. However the results of PRNT with these sera were not equivalent to IFA results because 3 out of 9 these sera contained high PRN antibodies against Hantaan virus and none of these 9 sera contained significant titers of PRN antibodies against Seoul or Puumala viruses. It remains to be studied more about the meanings of presence of IF antibodies to Puumala virus in sera from suspect HFRS patients who did not have antibodies to Hantaan virus in Korea. It is our desire to isolate the etiologic agent from bloods of these suspect HFRS patients for better understanding of etiology of HFRS and antigenic relations of hantaviruses. It is a speculation that there are at least one or two more new serotypes of hantavirus that cause HFRS or HFRS-like illness in Korea..

CONCLUSION

1. Nos. of HFRS patients confirmed at Institute of Viral Diseases, in Korea in 1990 and 1991 were 537 and 374 respectively. However, total nos. of HFRS patients confirmed serologically at 3 institutions in Korea (Institute of Viral Diseases, Korea University, Seoul National University Medical College and NIH, Korea) were 1,043 in 1990 and 956 in 1991.
2. Nos. of HFRS patients among Korean soldiers in 1990 and 1991 were 74 and 44 respectively. These figures are smallest since 1960 although the no. of Korean soldiers stationed at the DMZ is almost same as previous years.
3. Large outbreaks of scrub typhus, murine typhus and leptospirosis occurred among civilian in 1990 and nos. of patients were 683, 198 and 140 respectively. Small outbreaks of scrub typhus and murine typhus were observed among civilian in 1991 and nos. of patients were 376 and 149 respectively.
4. More than 50% of patients among suspect HFRS in 1990 and 1991 was unknown etiology and it remains to be studied further because there were some serological evidences of Colorado tick fever and Rocky Mountain spotted fever-like illnesses in Korea.
5. Male is dominant group in HFRS, murine typhus, spotted fever and leptospirosis but female is dominant group in scrub typhus.
6. Epidemic season of HFRS and scrub typhus is fall, October-December, and cases occur not only in rural areas but also in urban cities in all over the S. Korea throughout year.
7. Serologic evidences for hantavirus infection in wild bats and wild birds were demonstrated for the first time in Korea, and seropositive bats and birds were Rhinolophus ferrum-equinum, Vespertilo abramus, Paradoxomis webbiana and Emberiza elegans.
8. A simple and rapid serologic diagnostic test for Puumala virus infection was developed by high density silicon particle agglutination.
9. Thin section electron microscopy detected the occurrence of numerous dense precipitates, typical inclusion bodies, a surface antigen layer, as well as Hantaan virion-like structures in the kidneys of patients who died during the acute phase of HFRS.
10. Comparative study of serologic diagnosis of sera from suspect HFRS patients and seroepidemiologic survey against different serotypes of hantaviruses by IFAT showed that prototype Hantaan virus, Puumala virus and a local strain of hantavirus should be used for correct serologic diagnosis of hantavirus infection in different parts of the world.

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